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The Genetics of Bacteria (E72-C4)

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This report covers the period from February 1, 1952 to October 20, 1952.

A. Summary

In Salmonella, the agent of genetic transfer (transduction) has been tentatively identified as bacteriophage. Under certain conditions, it appears that phage particles may incorporate fragments of the genetic material of the host bacterium. When these phages infect a second host they may transduce these genetic factors to it. In previous studies, the genetic markers involved nutritional, fermentative and drug-resistance factors. Special attention has now been given to the transduction of flagellar antigens, which are the basis of serotypic classification in this group. Several new serotypes have been built up, as well as presently recognized species, by the recombination of antigenic factors: for example, S. paratyphi B (b: 1, 2) from S. typhimurium (i: 1, 2) X- S. abony (b: enx). The genetic basis of nonflagellated O-forms and of monophasic Salmonella types has also been explored, and should lead to useful applications in the simplified preparation of diagnostic sera and in the typing of otherwise untypable isolates. The genetic mechanism of antigenic phase variation has also been studied.

In E. coli, the genetic and physiological control of "sexual" fertility have been further explored. Stocks showing extremely high frequencies of recombination have been found, and some preliminary studies have been made on the cytological basis of recombination. These do not yet point to any definite conclusion. Lysogenic bacteriophage has been found to behave as if it were a genetic factor, and an isolated instance of genetic transduction by phage has been found. These observations provide further support for the previously unsuspected genetic functions of symbiotic phages. Immunogenetic studies with different fertile lines of E. coli have shown that serotypic factors are under direct genetic control much as in higher organisms.

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B. Full statement of progress

1. Salmonella.

Previous studies summarized in the last report (February, 1952) have brought to light a new mechanism of genetic exchange in Salmonella typhimurium: genetic transduction. Various hypotheses have been entertained on the nature of the transducing agent, including the possibility of its relationship to "L-forms," but were abandoned as new evidence was developed. The agent (FA--filtrable agent) was most consistently found in lysates provoked by phage and appeared to be associated with particles about 0.1 micron in diameter. These particles are evidently bacteriophage itself. The identity of FA with phage has been supported by the similarity of FA and phage titrations after filtration through gradocol membranes, high speed centrifugation, and most pertinent, adsorption on specific Salmonella hosts. It has not yet been proven that the same particles which transduce can also lyse; possibly, the transducing particles represent immature or imperfect phage. However, the more general question has not been settled what determines whether a bacterium will lyse or will become symbiotically infected after it adsorbs a particle of the "temperate" phages used in these investigations. Whether lytic phages also potentially transduce cannot be tested as the new host cells will be destroyed. Whether temperate phages in other bacterial species may have similar genetic functions is a point that we hope to examine when the occasion permits.

Aside from the theoretical implications of the mediation of genetic transfer by phage, the characterization of FA has been very important in guiding the preparation of FA from other Salmonella serotypes for studies on antigenic recombination. The host range of the phage that has been used in most of the studies to date, PLT-22, is confined to serotypes carrying the XII2 somatic antigen, i.e. most members of groups B and D. Temperate phages with different or overlapping host ranges would be useful to extend the range of these experiments, especially if one could apply a phage with cosmopolitan range, specific for the "R" antigens. Dr. C. C. Spicer (supported by a W.H.O. fellowship) is currently working with the principal investigator, but the phages tested have not yet been brought to a sufficiently high titre.

FA has been prepared from a considerable number of serotypes in groups B and D, and investigated in respect to the basis of O-forms, monophasic types, the mechanism of phase variation, and the synthesis of new serotypes.

The studies on O-forms were carried out in association with Dr. B. A. D. Stocker (a Commonwealth Fund fellow). Salmonella types lacking flagella and H antigens were received from several sources. In some cases, they were known to originate from a definite type; the history and type of others were unknown. In addition, a phage was received from N. Boulgakow (Paris) that had been described in 1936 as specific for flagellated species of Salmonella. This old report had been forgotten or discredited, but we were able to confirm his principal findings. The flagellotropic phage has been a convenient reagent for selecting nonflagellated O forms from the occasional susceptible H strains. There is no obvious relationship between the flagellar serotype and susceptibility.

FA prepared from motile Salmonellas was applied to various O forms in semi-solid agar, so as to select for any transductions of motility. Swarms of motile bacteria were obtained in every case. Wherever the original type was known, the transinduced motile forms conformed to it, rather than to the source of the FA.

In another instance, the serodiagnosis of the transinduced H form agreed with previous suspicions from the biochemical behavior of the original 0 (S. dublin--P. R. Edwards, priv. comm.). One exception to this rule was found in a strain provided by F. Kauffmann, an 0 form described as originating from a monophasic S. paratyphi b. The motile swarms obtained from this 0 form were of two types: the first b, conforming with the original strain, and the second conforming to the source of the FA e.g., i from S. typhimurium. Further genetic studies on these i transinductions support the conclusion that they are exceptional transfers of two closely linked factors, one for motility, the other for the specificity of the H antigen whereby b is substituted by i.

Several genetic loci are concerned in the immotility of different 0 forms. FA was prepared from each of them, whenever feasible, and applied to each of the others. Each FA conferred motility on each of the other 0 forms, showing that each 0 form could complement the genotype of any other. No 0 form could transduce itself, but FA from spontaneous motile reversions (which occur infrequently in the strains examined) conferred motility on the parent 0. At least five or six loci for flagella have thus been identified, but only one of them is linked to a determinant of H antigens. It seems reasonable that hitherto untypable strains should be typed by transducing motility to them from a motile Salmonella. A rare flagellar type should be used as the source of FA to facilitate the detection of exceptional cases where the transinduced H may conform to the FA rahter than its own original type. Efforts to transduce motility to the natural 0 serotype, S. pullorum-gallinarum, were unsuccessful. This may be due partly to the strong lytic action of the phage on these hosts. In addition, the immotility may be due to the coincidence of several genetic changes which cannot be restored all at once by transduction.

Another category of immotile cultures was received from E. Leifson--these are "paralysed," i.e., they carry a distinctive H antigen (S. typhimurium) but the flagella are nonfunctional. FA from 0 forms conferred normal motility on the paralysed strains, and vice versa. In addition, two paralysed strains were found that were complementary to each other. It is concluded that two additional loci, distinct from these characterized in the 0 forms, are involved in flagellar function. Thus, genetic control over Salmonella flagella is exercised at three levels: (1) the determination of whether flagella will be formed at all (several loci), (2) whether they will function in motility (two loci) and (3) their antigenic constitution (one locus so far). The genetic loci involved appear to be entirely separate except for an unusual "linkage" of one of the factors of the first group with the third. Subsequent studies have been concerned primarily with immunogenetic problems. It may be pointed out, if further evidence needs to be adduced, that these genetic studies are entirely inconsistent with Pijper's theory of motility, according to which the flagella are accidental concomitants of motion, rather than the locomotor organs themselves.

Monophasic strains of Salmonella types have been recognized as useful reagents for the preparation of diagnostic antisera which will be uncontaminated by antibodies for alternative phases. Several monophasic cultures have been collected from the field by Edwards and Kauffmann, and are used for this purpose. It appears likely that monophasicity is controlled by the genetic background of the strain, rather than by the particular antigenic factor involved. This is borne out by the transductions numbered 1-10. All antigenic transductions to the monophasic

S. typhi, or to a monophasic variant S. paratyphi B have resulted in types which are themselves monophasic. If the technique can be extended to species carrying very infrequent somatic antigens, the transinductions should be especially useful for the preparation of diagnostic sera which will be effectively pure without absorption.

Work has been initiated on the genetic mechanism of phase variation. A consideration of transductions # 11 and 12, together with other data, has led to the following paradox: when a culture is in the specific phase, the antigenic factors for the second phase cannot be detected either in the FA from this phase, or by its serological reactions. The second phase is, however, latent in the bacteria because, for example in # 11, after b has been substituted for i, the second phase continues to be 1, 2. The same argument holds for the latency of the specific phase, conversely. The following hypothesis is suggested: phase 1 and phase 2 are determined by different factors at each of two loci, respectively. The functioning of the two factors is mutually exclusive, so that one locus is somehow suppressed. In a comparable situation in Paramecium, Sonneborn has shown that the expression of latent serotypes is a matter of the cytoplasmic state, but it appears more likely here to concern a mechanism more closely connected with the locus itself. There are few analogies for this behavior in the genetics of higher forms, except that many workers have made similar speculations for the differential functioning of different genes at different times and places during embryonic development. Further studies may be expected to furnish a solid basis for the confirmation or refutation of this hypothesis.

As a by-product of these studies, many new serotypes have been reconstructed, as shown in the accompanying table. In addition, familiar serotypes such as S. paratyphi B have been recovered, as in # 11. The principal investigator is planning to initiate a more systematic program of development of Salmonella types in collaboration with Dr. P. R. Edwards at the Public Health Service Communicable Diseases Center at Chamblee, Ga., in the course of a forthcoming visit to his laboratory. New Salmonella serotypes are being isolated from the field nearly every month. That transduction plays a likely role in the evolution of these types seems almost certain: the gut environment is known potentially to contain all of the essential elements--Salmonella bacteria, phages and copro-antibodies--of the laboratory model. It may also be argued a forteriori that care should be taken against the indiscriminate use of antibiotics in chemotherapy or in nutrition which may encourage a similar diffusion of drug-resistance from resistant variants that may be innocent in themselves to more serious pathogens inhabiting the same environment.

2. Escherichia coli.

The discovery of the genetic control of compatibility was outlined in the previous report. By an extension of this work, special combinations of parents have been shown to give a recombination frequency of as high as ten percent in four hours of mixed culture. This is still a relatively low rate for direct cytological observations, especially as optimal rates of recombination are associated with high rates of growth, which make it difficult to follow individual cells under the microscope. Some observations have been made from which high frequency strains (Hfr) seem to be associated with a poorly staining material that forms blebs or droplets near the cell surface. In a few instances, cells have been seen under phase microscopy that appeared to be stuck together, as if by this material, but it has not yet been possible to work out their subsequent behavior either in living

or in nuclear stained preparations. No suggestions of holocellular fusion or copulation have been seen. As a working hypothesis, it is suggested that the parent cells become temporarily attached to each other, and that a nucleus is transmitted from one to the other, followed by the separation of the parent cells. This would be comparable to the conjugation observed in ciliates, and consistent with the genetic finding that the zygotes appear to occur singly, even though the parent cells are presumably multinucleate. Dr. T. C. Nelson and the principal investigator are studying the physiological conditions for optimal frequencies of mating with the Hfr cultures in order to facilitate further cytological study.

Dr. E. M. Lederberg (Project Associate, Chemical Corps support) and a graduate student, M. L. Morse, are studying the genetic functions of bacteriophage in E. coli. The latent phage is transmitted in crosses as if it were attached to a definite locus in the bacterium. Conversely, transduction by this phage has been observed, but only for one marker--a locus very closely linked to the latent phage in crosses. Unlike Salmonella transduction, no other genetic factors appear to be transmissible by this latent phage.

The cross-fertility of new isolates of E. coli has permitted the development of an immunogenetic program with this organism (Dr. P. D. Skaar). O, K, and H reagent sera have been developed for several strains. The results of crosses so far show that different genetic factors control each of these major antigens, and new combinations are produced by crossing. Unfortunately, the E. coli lines of greatest medical interest (O111:B4 and O55) have not yet given positive results in crossing tests. Susceptibility to the well known T phages appears to be associated with the absence either of the O or of the K component.

REFERENCES

(a) Other workers

Sertic, V. and Boulgakov, N. A. 1936 Bacteriophages specifiques pour les varietes bacteriennes flagelles. C. R. Soc. Biol. 123: 887-888.

Edwards, P. R. and Bruner, D. W. 1946 Notes on monophasic Salmonella cultures and their use in the production of diagnostic serums. J. Bact. 52: 493-498.

Friewer, F. and Leifson, E. 1952 Non-motile flagellated variants of Salmonella typhimurium. J. Path. Bact. 64: 223-224.

Kauffmann, F. Enterobacteriaceae Munksgaard: Copenhagen, 1951.

Sonneborn, T. M. 1950 The cytoplasm in heredity. Heredity 4: 11-36.

C. Significant accomplishments

1. The discovery of genetic recombination in E. coli strain K-12, and in a number of new isolates.

2. The discovery of genetic transduction in Salmonella.

3. The demonstration that drug-resistance results from spontaneous mutation. Fuller detail is given in this and preceding progress reports, and in the following articles of which reprints have been furnished:

Lederberg, J. et al. 1951 Cold Spring Harbor Symposium 16: 413-443
"Recombination analysis of bacterial heredity"

Lederberg, J. and Lederberg, E. M. 1952 Jour. Bact. 63: 399-406
"Replica plating and indirect selection of bacterial mutants"

D. Plans for next year

For the immediate future, the following program is planned:

1. Physiological study of conditions of "sexual" recombination in E. coli, and cytological study of its material basis.

2. The relationship between the establishment of lysogenicity and genetic transduction following infection by temperate phages in Salmonella and E. coli.

3. The extension of transduction technique to other serotypes of Salmonella, especially for alterations from rough to smooth, and for substitutions of somatic and Vi antigens.

4. The development of a systematic program for breeding Salmonella types, especially those useful as antigens for diagnostic work.

5. The investigation of mechanisms of genetic exchange in other bacteria.

6. Problems of gene action in relation to the formation of specific enzymes and antigens.

Intertype transductions in Salmonella

	Transducer		Transducee	Result
1.	IV, V, XII; i: 1, 2 typhimurium	--X	IX, XII; d: - typhi	IX, XII; i: - new type
2.	IV, V, XII; b: 1, 2 paratyphi B	--X	IX, XII; d: - typhi	IX, XII; b: - new type (monophasic S. onarimon)
3.	IV, XII; c: 1, 7 altendorf	--X	IX, XII; d: - typhi	IX, XII; c: - new type
4.	IV, V, XII; r: 1, 2 heidelberg	--X	IX, XII; d: - typhi	IX, XII; r: - new type (monophasic S. shoreditch)
5.	IV, V, XII; eh: enz15 sandiego	--X	IX, XII; d: - typhi	IX, XII; [redacted]5 eh new type
6.	IV, V, XII; i: 1, 2 typhimurium	--X	IV, V, XII; (b): -- paratyphi B, O form monophasic	IV, V, XII; i: - typhimurium, monophasic
7.	IV, XII; c: 1, 7 altendorf	--X	IV, V, XII; (b): --	IV, V, XII; c: - altendorf, monophasic
8.	IV, V, XII; r: 1, 2 heidelberg	--X	IV, V, XII; (b): --	IV, V, XII; r: - heidelberg, monophasic
9.	I, IX, XII; gm: - enteritidis	--X	IV, V, XII; (b): -	IV, V, XII; gm: - ossen
10.	I, IX, XII; (gp): dublin O form	--X	IV, V, XII; (b): -	IV, V, XII; gp: - new type
11.	I, IV, XII; b: enx abony	--X	IV, V, XII; i: 1, 2 typhimurium	IV, V, XII; b: 1, 2 paratyphi B
12.	I, IV, XII; b: enx abony	--X	IV, V, XII; i: 1,2 typhimurium	IV, V, XII; i: enx new type